

What is claimed is:

1. A method of enriching chaperone protein complexes from a sample comprising chaperone protein complexes, said method comprising the steps of
5 (a) subjecting the sample to free solution isoelectric focusing; and
(b) collecting one or more fractions with a pH from pH 4.5 to 6.5 which are enriched in chaperone protein complexes.

10 2. The method of claim 1, wherein said free solution isoelectric focusing is performed in the presence of a non-ionic or zwitterionic detergent and urea at a concentration in the range of 4M-8M.

15 3. The method of claim 1, wherein said step of subjecting the sample to free solution isoelectric focusing comprises
(i) forming a pH gradient that comprises a pH value in the range from pH 4 to pH 7 in a matrix comprising charged buffer particles; and
(ii) electrophoresing the sample through the pH gradient in the matrix for a period of time until the chaperone protein complexes cease to migrate within the pH gradient.
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4. The method of claim 3, wherein said matrix is comprised of a non-ionic or zwitterionic detergent and urea at a concentration in the range of 4M-8M

25 5. The method of claim 4, wherein the urea is at a concentration in the range of 4M-8M.

6. The method of claim 4, wherein the detergent concentration is in the range of 0.1%-1.7%.

30 7. The method of claim 4, wherein the detergent is zwitterionic.

8. The method of claim 4, wherein the detergent is non-ionic.

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9. The method of claim 3, wherein said matrix comprises 6 M urea, 0.5% Triton X-100, 0.5% Triton X-114, 0.5% Igepal CA-630, 5 mM Tris/Cl (pH 7.4), and 5 mM NaCl.

5 10. A method of making a composition enriched with chaperone protein complexes from a plurality of cells, said method comprising the steps of :

- 10 (a) making a cell lysate by lysing the cells with a buffer consisting of 10 mM Tris/Cl, 10 mM NaCl, 0.1% Triton X-100, 0.1% Triton X-114, 0.1% Igepal CA-630, 2 μ g/ml leupeptin, 1 μ g/ml pepstatin A, and 0.5 mM phenylmethylsulfonyl fluoride;
- 15 (b) dialyzing the cell lysate from (a) into a buffer consisting of 5 mM Tris/Cl, 5 mM NaCl, 0.05% Triton X-100, 0.05% Triton X-114, 0.05% Igepal CA-630;
- (c) dialyzing the cell lysate from (b) into a buffer consisting of 2.5 mM Tris/Cl, 2.5 mM NaCl, 0.025% Triton X-100, 0.025% Triton X-114, 0.025% Igepal CA-630;
- (d) dialyzing the cell lysate from (c) into a buffer consisting of 1.25 mM Tris/Cl, 1.25 mM NaCl, 0.012% Triton X-100, 0.012% Triton X-114, 0.012% Igepal CA-630;
- 20 (e) dialyzing the cell lysate from (d) into water;
- (f) adding the cell lysate from (e) to a solution comprising urea and ROTOLYTES®;
- (g) forming a pH gradient that comprises a pH value in the range from pH 4 to pH 7 in a buffer comprising 6M urea, 0.4% Triton X-100, 0.4% TritonX-114, 0.4% Igepal CA-630 and 30 ml of ROTOLYTES®;
- 25 (h) electrophoresing the cell lysate (f) through the pH gradient in the matrix with a voltage of 500 to 2000 volts for 5 hours;
- (i) collecting one or more fractions with a pH from pH 4.5 to 6.5; and
- (j) dialyzing the fractions against a solution comprising phosphate buffered saline.

30 11. A pharmaceutical composition comprising a sample enriched in chaperone protein complexes, and a pharmaceutically acceptable excipient, wherein the sample is prepared by a method comprising

- 35 (a) subjecting a solution comprising chaperone protein complexes and a plurality of different proteins to isoelectric focusing, and

(b) collecting one or more fractions with a pH from pH 4.5 to 6.5; wherein at least some of the proteins in the solution are present in fractions other than fractions of pH 4.5 to pH 6.5; wherein the collected fractions comprise a mixture of chaperone protein complexes;

(c) wherein said chaperone protein complexes in said sample are not purified to homogeneity.

12. The composition of claim 11, wherein the isoelectric focusing is performed in the presence of urea and detergent.

13. The composition of claim 11, wherein the isoelectric focusing is performed in the presence of urea at a concentration in the range of 4M-8M.

14. The composition of claim 11, wherein the isoelectric focusing is performed in the presence of detergent at a concentration in the range of 0.1%-1.7%.

15. The composition of claim 11, wherein the isoelectric focusing is performed in the presence of detergent that is zwitterionic.

16. The composition of claim 11, wherein the isoelectric focusing is performed in the presence of detergent that is non-ionic.

17. The composition of claim 11, wherein the isoelectric focusing is performed in the presence of detergent that is 0.1% Triton X-100, 0.1% Triton X-114, and 0.1% Igelpal CA-630.

18. The pharmaceutical composition of claim 11, wherein said sample comprises pooled proteins from said collected fractions.

19. The pharmaceutical composition of claim 11, wherein the chaperone protein complexes are present in aggregates that have a molecular weight that is greater than 300 kD.

20. The pharmaceutical composition of claim 19, wherein the aggregate chaperone protein complexes comprise GRP94/gp96, HSP 90, HSP70, calreticulin, BiP/grp78, grp75/mt, HSP 70, HSP72, HSP60, and HSP40.

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